Furthermore, we show that both the single known Aplnr ligand, Apelin, and the canonical Gαi/o proteins that signal downstream of Aplnr are dispensable for Aplnr function. This work suggests a novel mechanism for Aplnr signaling in the establishment of a niche required for the proper migration/development of myocardial progenitor cells. Current work is focused on determining the alternate fate or location of cells destined for the heart-forming region in the absence of Aplnr signaling and when migration of these cells goes awry. The non-autonomous cue mediated by Aplnr signaling is also being investigated.

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Program/Abstract # 280
Cardiac BAF complex promotes heart progenitor differentiation and migration in the zebrafish embryo
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Congenital heart defects and adult-onset heart disease are among the most critical health problems in developed countries. A greater understanding of cardiac progenitor biology will ultimately be essential for regenerative and early-intervention therapeutic applications. At the onset of cardiomyocyte differentiation, a cardiac progenitor-specific gene expression module must somehow be initiated. This initial event must depend on appropriate epigenetic regulation activities, including chromatin-remodeling, which can modify DNA-histone interactions, thereby changing the availability of transcription factor binding sites. In eukaryotes, the BAF (Brg1 associated factor) complexes are large multi-subunit protein assemblies with chromatin-remodeling activities. These complexes can engage in a number of cell-specific events via differential use of variant subunits. Expression of Baf60c, a cardiac specific subunit of the BAF complex, with Gata4 and Tbx5 is sufficient to promote differentiation of mesodermal cells to cardiomyocytes in murine embryos. To uncover the endogenous role of this cardiac BAF (cBAF) complex in cardiac progenitors, we have used the zebrafish model. We first transplanted cells overexpressing gata5/baf60c to a wildtype host and found these cells could spontaneously migrate to the heart-forming region and contribute to myocardium, endorcardium and smooth muscle at outflow tract. Remarkably, this occurred independent of the location cells were placed in the host. Further transplantation experiments using hosts with defects in various germ layers indicate that signal(s) emitted from the endoderm is dispensable for cBAF complex-driven cardiac progenitor migration and differentiation. Global overexpression of these three genes elevated the expression of heart-specific genes, resulting in an enlarged heart. In a fish embryonic cell culture/induction system the overexpression of cBAF also promoted differentiation of contractile cardiomyocyte. To determine the endogenous function of cBAF, baf60c together with gata5 and tbx5 were knocked down in the zebrafish embryo through morpholino injection. This led to massive downregulation of myocardial gene expression, with the morphants displaying severe heart defects. The ultimate fate of cBAF cells can be modulated, as shown by Fgf signaling inhibition leading to decreased myocardial, but not endocardial, differentiation of cBAF cells. Therefore, cBAF (Gata5/Smarcd3b) can promote formation of cells that home to the heart-forming region regardless of inhibitory signals in the embryo. As these cells can form all the lineages of the developing heart, these results show that cBAF can drive, in vivo, a CPC-like state.

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Program/Abstract # 282
Investigating an interchangeable potential between heart and gut mesothelial development
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The mesothelium is an epithelial sheet that covers organs in the coelomic cavity and is involved in development of the vascular system. In the developing heart, the proepicardial organ (PE), migrates to and over the heart to form the epicardium, and undergoes epithelial–mesenchymal transition (EMT) to give rise to the cells of the coronary blood vessels. The gut mesothelium (GM) serves as a major source of vascular smooth muscle cells for the gut tube in development. The role of mesothelial cells in both the heart and gut in the formation of blood vessels suggest that there may be similarities, possibly an interchangeable potential, in mesothelial development that exists among coelomic organs. To test the interchangeable potential of mesothelial cells, we used the chick-quail chimera system to transplant quail PEs into the peritoneal cavity of a chick embryo and quail GM cells into chick pericardial cavities. Our initial findings have revealed that both cell types have the potential to migrate into organs in the coelomic cavities, but PE cells do not incorporate into the endogenous GM, while GM cells will incorporate into the endogenous epicardium. However, in both systems, transplanted PE and GM cells become positive for smooth muscle. Taken together, our current data suggest that although the epicardium and GM appear similar in structure in the embryo and adult, and can potentially give rise to smooth muscle actin positive cells, we observe fundamental differences in how the mesothelium develops in the heart and gut.

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Program/Abstract # 283
The role of ß-catenin and Eomesodermin in the establishment of progenitor and stem cell lineages during intestinal endodermal development
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Many disorders are a direct result of the impaired development of the primitive gut tube, which is derived from the endodermal germ layer. Although the canonical Wnt pathway is central for many developmental processes, few studies have examined Wnt and its key player, β-catenin, in early endoderm specification. Similarly, Eomesodermin has only recently been implicated in definitive endoderm development. In addition, many of the genes that, in articulation with Eomesodermin, orchestrate trophectoderm lineage establishment in the early embryo, including Nodal, Cdx2, Fgf4 and Ascl2, will later prove decisive in initiating posterior endodermal fates and intestinal identity. In order to determine the role of β-catenin and Eomesodermin in the regional specification of gut endoderm progenitors, we designed a novel approach combining Cre-mediated mutagenesis and

experimental explants. To overcome the embryonic lethality of both β-catenin and Eomesodermin mutant embryos, we crossed mice with conditional (floxed) β-catenin stabilized, β-catenin null and Eomesodermin null alleles, with the inducible Cre-driver mouse line, Claudin-6CreERT2 which is specific to the endodermal compartment. Also, by tuning the tamoxifen dosage, mosaic recombination will be enabled by the CreERT2 system, and used for clonal studies. At present, we are conducting an exhaustive phenotypical analysis in conjunction with biochemical studies and the establishment of in vitro organoid cultures from mutant and control mice. Disclosing the transcription factor networks involved in endoderm and intestinal progenitor specification is absolutely critical to a better understanding of the ontogeny of various gut diseases, and will encourage the development of novel therapeutics, considerably improving human health.

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Program/Abstract # 284
Zebrafish mnx1 controls cell fate choice in the developing endocrine pancreas

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In type-1 diabetic patients glucose homeostasis is regulated by frequent injections of insulin. Beta cell mass can be restored by islet transplantation, but lack of organ donors and the requirement for immunosuppressants make this approach problematic. Recent developments in protocols to differentiate embryonic stem cells to beta cells provide hope for a renewable source of transplantable beta cells. In vertebrates Pdx1-expressing endoderm cells give rise to endocrine and exocrine cell lineages. In previous work we established that development of all pancreatic lineages requires retinoic acid (RA) signaling upstream of Pdx1. However, the gene regulatory network that leads to endocrine pancreas specification downstream of RA signaling is not well characterized. Using an unbiased microarray approach we identified Mnx1 (Hb9) as a RA regulated endoderm transcription factor. By combining manipulation of gene function with transgenic reporter analysis we establish a critical role for Mnx1 in controlling cell fate decisions within the endocrine pancreas progenitor lineage. Previous studies in both zebrafish and mouse have revealed that Mnx1 plays a role in beta cell development. Here, we show that RA signaling regulates the expression of mnx1 in the endoderm, and that mnx1 functions downstream of RA signaling to establish beta cell fate. Published work has shown that morpholino-mediated knock down of zebrafish mnx1 decreases beta cell number. We now demonstrate that in Mnx1-deficient embryos beta cell precursors fail to express insulin and convert to glucagon expressing alpha cells. In summary, we propose that mnx1 functions downstream of RA signaling in the endocrine progenitors to promote beta cell fate.

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Program/Abstract # 285
Mechanism of genetic interaction between Hnf1b and Wnt/ß-Catenin signaling for specification of hepatopancreatic progenitors

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Maturity onset diabetes of the young 5 (MODY5) is a monogenic form of juvenile diabetes caused by heterozygous mutations in the gene encoding the transcription factor HNF1B (Hepatic Nuclear Factor 1B). While MODY5 patients commonly present with a hypoplastic pancreas, it remains unclear how mutations in HNF1B affect developmental of the pancreas or cause diabetes. Currently, only animals with extreme loss of function Hnf1b alleles have been studied, but these models exhibit severe endoderm regionalization defects that preclude analysis of pancreas development and function. We have now identified a novel zebrafish mutant with a partial loss of function mutation in hnf1b that phenocopies MODY5 pancreatic defects. Using this novel hnf1b mutant, we have uncovered a synthetic genetic interaction with Wnt/β-Catenin signaling in specifying hepatopancreatic progenitors. We are using gain and loss of function experiments to determine the mechanism of their genetic interaction. Our studies suggest that Hnf1b is necessary for Wnt/β-Catenin activity to induce hepatopancreatic development in the endoderm. This work has profound implications for diabetes etiology and may provide insight into the molecular events necessary for in vitro generation of therapeutic pancreas and liver cells.

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Program/Abstract # 286
Grg3 corepressor is required for the differentiation of secondary transition endocrine cells in the embryonic pancreas

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There have been increasing efforts to generate insulin-producing beta-cells in culture for therapeutic uses, drawing on the biological concepts learned from developmental biology. However, these cultures have yet to reproducibly produce glucose-responsive, mature beta-cells, indicating that a greater understanding of the developmental processes to generate fully functional beta-cells is needed. Grg3 (Groucho Related Gene 3) is a member of the Groucho/TLE family of corepressors which facilitates repression of gene expression, and its function in various cell contexts are mediated by its recruitment to target genes by different transcription factors. Grgs broadly regulate the progression of progenitor cells to differentiated cell types and direct cell fate decisions during development. We have found that Grg3 is expressed in most beta-cells and a subset of other endocrine cell types in the fetal and adult mouse pancreas. We have also determined by Ngn3-Cre driven cell lineage tracing experiments that Grg3 is activated in Ngn3+ endocrine progenitor descendants just after transient Ngn3 expression. Analysis of Grg3 knockout embryos determined that a smaller but morphologically normal pancreas (including primary transition glucagon cells) is formed by E13.5. However, the bulk of endocrine differentiation known as the secondary transition occurs around E14, but the vast majority of Grg3 knockout embryos die before E14.5 because of placental defects. Therefore, we explanted E12.5 pancreata to allow differentiation to occur in culture. While control explants displayed all aspects of pancreatic differentiation including exocrine (amylase), ductal (muc1) and endocrine (insulin, glucagon) differentiation, Grg3 knockout explants displayed a drastic decrease (~85% decrease) in endocrine cell